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Antibacterial activities of essential oils from aromatic and medicinal plants against growth of phytopathogenic bacteria

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Abstract. In search for alternative ways of plant disease control, essential oils from aromatic and medicinal plants were studied for their antibacterial properties. In a preliminary evaluation, steam-distilled essential oils from oregano, sweet-flag, caraway, peppermint, common, fern leaf and willow-leaved yarrow field accessions were investigated against growth of phytopathogenic bacteria. The disc-diffusion method was used for the assessment of inhibitory effects of the essential oils. The antibacterial activity tests were conducted *in vitro.* Essential oil from oregano was found to have the strongest inhibitory effect against tested phytopathogenic bacteria. Less antibacterial effect was found with essential oils from caraway, peppermint, fern-leaf and willow-leaved yarrow,, while the oils from common yarrow and sweet-flag manifested no inhibition. *Xanthomonas vesicatoria* 67 was the most sensitive to essential oils tested. A weak antibacterial activity was found against some *Pseudomonas* spp. strains and *Erwinia carotovora* subsp. *carotovora* bacteria.

Key words: essential oils, phytopathogenic bacteria, antibacterial activity

INTRODUCTION

Bacterial pathogens and their control are a serious problem in agriculture practice. Spraying with antibiotics and copper compounds, usually suggested to control bacterial diseases, have never been satisfactory. Furthermore, antibiotics are forbidden in many countries and copper compounds, because of their general toxicity, exert a negative impact on both yield and the environment. As an alternative strategy to prevent the spread of diseases, natural compounds of plants are being tested for their antimicrobial activity. Naturally occurring biologically active plant products can be a source of new pesticides or serve as templates for new, more effective compounds (Elkovich, 1988).

Investigations of aromatic and medicinal plants enable finding plants producing effective essential oils that have already found a considerable range of applications. Various essential oils are biocides against a broad range of organisms such as bacteria, fungi, viruses, protozoa, insects and plants (Kalemba & Kunicka, 2003).

In recent years a large number of essential oils and their constituents have been investigated for their antimicrobial properties against bacteria and fungi. There is vast diversity among aromatic and medicinal plants. Different chemotypes of the same species may grow in the same place and produce different oils with different activity (Kalemba & Kunicka, 2003). The current work presents an evaluation of antibacterial

activity of essential oils from aromatic and medicinal plants of Lithuania and their inhibitory effect against the growth of phytopathogenic bacteria.

MATERIALS AND METHODS

Essentials oils were isolated by hydrodistillation from the air-dried plant material from oregano (*Origanum vulgare* L.) sweet-flag (*Acorus calamus* L.), common, fern-leaf and willow-leaved yarrow (*Achillea millefolium* L., *A. filipendulina* L., *A. cartilaginea* L.), caraway (*Carum carvi* L.), peppermint (*Mentha x piperita* L.) field accessions growing at the Institute of Botany /Vilnius, Lithuania/. The composition of essential oils was investigated using gas chromatography and mass spectrometry analysis (GC and GC–MS). Extracted oils were tested against strains of phytopathogenic bacteria. *Erwinia carotovora* subsp. *carotovora* 1122, *Xanthomonas vesicatoria* 67, *Pseudomonas marginalis* pv. *marginalis* 1763, *P. syringae* pv. s*yringae* 1139, *P. syringae* pv. s*yringae* 1, *P. syringae* pv. *tomato* 506, *Bacillus* sp. 1044.

The antibacterial activity tests were conducted *in vitro.* Bacteria were isolated from injured plants and reference strains *Pseudomonas marginalis* pv. *marginalis* obtained from the Göttingen Collection of Phytopathogenic Bacteria /GSPB, Göttingen, Germany/ and *Xanthomonas vesicatoria* 67 from the Institute for Epidemiology and Resistance in Aschersleben, Germany. The inhibitory effect of essential oils was tested using the disc-diffusion method (Klement et al., 1990).

Bacterial suspensions were prepared of 24 h old cultures 10^8 cfu/ml and Petri dishes inoculated with 0.1 ml. Filter paper discs (5mm in diameter) were immersed in the undiluted oils and placed onto the inoculated potato dextrose agar medium. Control treatments consisted of streptomycin solution 0.02% and sterile-distilled water. Three discs were placed on each plate and each treatment had 2 plates. The plates were incubated at 27^{o}C for three days, then examined and the results recorded (Kalemba & Kunicka, 2003).

The inhibitory effect of the treatment against each test bacterium was determined by measuring the diameter of zones of inhibition (in millimetres) excluding paper discs.

RESULTS AND DISCUSSION

Essentials oils used in the paper diffusion assay varied in their antibacterial activity. Significant differences were evident among effects of essential oils from oregano and the other plants (Table 1). Essential oils from oregano had the strongest inhibitory effect against tested bacteria. Investigations on chemical composition of essential oils showed high variability among *O. vulgare* accessions and influenced differences in their antibacterial activity. Mono- and sesqiuterpene hydrocarbons were the dominant components in essential oils of oregano in Lithuania. Oils were represented mainly by β-caryophyllene, cis- and trans-β-ocimene, sabinene and germacrene. Investigated essential oils contained very low amounts of carvacrol and thymol (Radušienė et al., 2005). According to the data of other authors the effect of carvacrol and thymol is responsible for different antibacterial activities of essential oils of a wide range of plants (Kalemba & Kunicka, 2003; Hevesi et al., 2005). The effect

of the investigated oils on phytopathogenic bacteria was of low significance and could be caused by other compounds, apart from carvacrol and thymol.

Essential oils from oregano showed significant activity on *X. vesicatoria* 67, *Pseudomonas syringae* pv. *syringae* 1139, *P. marginalis* pv. *marginalis* 1763 *and Bacillus* sp. 1044 strains (Table 1). Oregano oils from different accessions were inactive or had a weak inhibition on soft rotting *Erwinia carotovora* subsp. *carotovora* 1122 and *Pseudomonas syringae* pv. *syringae* 1 bacteria.

Table 1. Antibacterial activities of plant essential oils on growth of phytopathogenic bacteria.

Essential oils		Inhibitory effect on bacterial strains						
Plant	accession No	1763	1	1139	506	1044	1122	67
Origanum vulgare	394 L	0	+	++++	+++	+++	+	++++
	394 F	0	+	++	++	++	+	+++
	281 F	0	+	+++	+++	+++	+	++++
	396 F	0	+	+++	++	+++	+	+++
	340 F	0	+	+++	++	+++	+	+++
	528F	+++	++	++	0	+	+	+++
	228L	++	–	–	0	+	–	++
	473L	+++	+	+	0	+	–	++
	311F	+++	–	+	0	+	–	++
Acorus calamus	12 L	–	–	–	–	–	–	–
	4 R	–	–	–	+	+	–	+
	13 R	–	–	–	–	–	–	–
	15 R	–	–	–	–	–	–	–
	18 R	–	–	–	–	–	–	–
	9 R	–	–	–	–	–	–	–
	7 R	–	–	–	–	–	–	–
	14 R	–	–	+	++	+	+	+
	17 R	+	–	–	+	+	–	–
	10 R	–	–	–	–	–	–	–
Achillea millefolium	513 F	–	–	–	–	–	–	–
	493 F	–	–	–	–	–	–	–
	377 F	–	+	–	–	–	–	–
Achillea filipendulina	389 F	–	–	+	+	+	+	++
	389 L	+	+	–	+	+	+	++
Achillea cartilaginea	228 L	–	+	++	+++	+	+	++
Carum carvi		–	–	–	–	–	+	++++
Mentha x *piperita*		–	+	–	+	+	+	++
Streptomycin (0.02%) K+		+	+	+	++	+++	+	++

*Bacterial strains: 1763 – *Pseudomonas marginalis* pv. *marginalis;* 1, 1139 – *P. syringae* pv. *syringae;* 506 – *P. syringae* pv. *tomato;* 1044 – *Bacillus* sp.; 1122 – *Erwinia carotovora* subsp. *carotovora;* 67 – *Xanthomonas vesicatoria*

*Diameter of inhibition zone: '++++'– 20 mm and more; '+++'– 12–20 mm; '++'– 6–12 mm; '+'– 2–6 mm; '–'– no antibacterial activity; 0 – not tested

*Essentials oils extracted from: L – leaves; F – flowers; R – rhizomes

Less inhibitory effect on separate bacterial strains was found in essential oils extracted from *Achillea filipendulina, A. cartilaginea, Carum carvi* and *Mentha x piperita* while no inhibitory activity of essential oils was manifested in *Achillea millefolium* and *Acorus calamus*. Recent investigations demonstrated high biological activity of *Acorus calamus* on phylamentous fungi and insects (Mungkornasawakul et al., 2002; Jiyavorranant et al., 2003), while no antibacterial effect was found against the growth of the examined phytopathogenic bacteria.

Among all tested bacteria *Xanthomonas vesicatoria* 67 was sensitive to the majority of essential oils. Essential oil from caraway (inhibition zone – 26 mm) exerted a particularly strong inhibitory activity on its growth.

CONCLUSIONS

The results showed varying effects of essential oils against phytopathogenic bacteria. The most effective in inhibiting the growth of bacteria was essential oil from oregano. The causal agent of tomato bacterial spot *Xanthomonas vesicatoria* 67 was the most sensitive to essential oils extracted from caraway and different accessions of oregano.

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